

In the specification

Please replace the paragraph on page 1, lines 5-8. with the following paragraph:

This application is a continuation of U.S. Serial No. 10/006,915 filed November 9, 2001,
which is a continuation of U.S. Serial No. 09/156,809 filed September 18, 1998 (now U.S. Patent
No. 6,316,262), which claims priority to U.S. Serial No. 60/059,373 filed September 19, 1997,
entitled Biological Systems for the Manufacture of Polyhydroxyalkanoate Polymers containing
4-Hydroxyacids by Gjalt W. Huisman, Frank A. Skraly, David P. Martin, and Oliver P. Peoples.

Please replace the paragraph on page 6, lines 16-21, with the following paragraph:

Figure 1A is the alignment of the *C. kluyveri* OrfZ sequence with the N-terminal
sequence and internal sequences of 4-hydroxybutyryl CoA transferase (4HBCT) from *C.*
aminobutyricum (SEQ ID Nos 1 and 2. Identical residues are indicated, similar residues are
indicated by *. ~~Figure 1B is~~ Figure 1B and Figure 1C are the nucleotide sequence of the *orfZ*
gene from *C. kluyveri kluyveri* (SEQ ID NO:3). ~~Figure 1D is~~ Figure 1D is the amino acid sequence of the
orfZ gene from *C. kluyveri kluyveri* (SEQ ID NO:1).

Please replace the paragraph on page 7, lines 9-12, with the following paragraph:

~~Figure 6 is~~ Figure 6 and Figure 6A are a schematic of the construction of plasmids for
integration of 3-ketoacyl-CoA thiolase (*phbA*) and acetoacetyl-CoA reductase (*phbB*) genes from
Z. ramigera into the chromosome of *E. coli* and other Gram-negative bacteria.

Please replace the paragraph bridging pages 22 and 23 with the following paragraph:

Plasmid pMUXC₅cat contains the *phbC* gene from *Z. ramigera* on a transposable element for integration of this gene on the chromosome of a recipient strain (Figure 5). Strong translational sequences were obtained from pKPS4 which encodes PHA synthase encoding *phaC1* from *P. oleovorans* in the pTrc vector (Pharmacia). In this construct, *phaC1* is preceded by a strong ribosome binding site: AGGAGGTTTTT(-ATG) (SEQ ID NO:4). The *phaC1* gene, including the upstream sequences, was cloned as a blunt ended *EcoRI-HindIII* fragment in the *SmaI* site of pUC18Sfi to give pMSXC₃. A blunt ended *cat* gene cassette was subsequently cloned in the blunt-ended *Sse8387II* site, resulting in pMSXC₃cat. At this point, all of the *phaC1* coding region except the 5' 27 base pairs were removed as a *PstI-BamHI* fragment and replaced by the corresponding fragment from the *phbC* gene from *Z. ramigera*. The resulting plasmid, pMSXC₅cat, encodes a hybrid PHB synthase enzyme with the 9 amino terminal residues derived from the *P. oleovorans* PHA synthase and the remainder from *Z. ramigera*. The C₅cat cassette was then excised as an *AvrII* fragment and cloned in the corresponding sites of pUTHg, thereby deleting the mercury resistance marker from this vector. The resulting plasmid, pMUXC₅cat, contains a C₅cat mini-transposon in which *phbC* is not preceded by a promoter sequence. Expression of the cassette upon integration is therefore dependent on transcriptional sequences that are provided by the DNA adjacent to the integration site.

Please replace the paragraphs on page 23, lines 25-29, with the following paragraphs:

3A (5'GGCTCGTATAATGTGTGGAGGGAGAACCGCCGGGCTCGCGCCGTT)

(SEQ ID NO:5) and

3B (5' CTAGAACGGCGCGAGCCCGGCGGTTCTCCCTCCACA CATTATACGA

GCCTGCA) (SEQ ID NO:6).

Please replace the paragraph bridging pages 23 and 24 with the following paragraph:

Next, a fragment containing a consensus *E. coli pho* box and -35 promoter region were inserted into the *PstI* site as a fragment obtained after annealing the oligonucleotides:

2A: (5' TCCCC TGTCATAAAGTTGTCACTGCA) (SEQ ID NO:7) and

2B (5' GTGACAACTTTATGACAGGGG ATGCA) (SEQ ID NO:8). Next, the messenger stabilizing sequence including the transcriptional start site from AB₅ was inserted into the *XbaI*-*NdeI* sites as a fragment obtained after annealing the oligonucleotides: 4A (5': CTAGTGCCGG ACCCGGTTCCAAGGCCGGCCGCAAGGCTGCCAGAACTGAGGAAGCACA)

(SEQ ID NO:9) and

4B: (5'TATGTGCTTCCTCAGTTCTGGCAGCCTTGCGGCCGGCCTTGGA

CCGGGTCCGGCA) (SEQ ID NO:10). The resulting plasmid is pMSXp₁₂AB₅kan2. The *AvrII* fragment, containing Tp₁₂AB₅kan2 was cloned into pUTHg cut with *AvrII* and used for integration into the genome of MBX379 and MBX245.

Please replace the paragraphs on page 30, lines 27-30, with the following paragraphs:

4HBD-N: 5 'CTCTGAATTCAAGGAGGAAAAAATATGAAGTTAT

TAAAATTGGC (*EcoRI*) (SEQ ID NO:11)

4HBD-C: 5 'TTTCTCTGAGCTCGGGATATTTAATGATTGTAGG

(*SacI*) (SEQ ID NO:12).

Please replace the paragraphs on page 31, lines 24-26, with the following paragraphs
(noting that a portion of the sequence in each of lines 24 and 26 was *underlined in the original*):

GH-Up: 5' AACGAATTCAATTCAGGAGGTTTTTATGGATCAGAC

ATATTCTCTGGAGTC (*EcoRI*) (SEQ ID NO:13)

GH-Dn: 5' TTGGGAGCTCTACAGTAAGAAATGCCGTTGG (*SacI*) (SEQ ID NO:14).

Please replace the paragraphs on page 31, lines 30-33, with the following paragraphs:

GB-Up: 5' TAAGAGCTCAATTCAGGAGGTTTTTATGGATAAGAA

GCAAGTAACGGATTTAAGG (*SacI*) (SEQ ID NO:15)

GB-Dn: 5' TTCCCGGGTTATCAGGTATGCTTGAAGCTGTTCTGT

TGGGC (*XmaI*) (SEQ ID NO:16).

Continuation of U.S.S.N. 10/006,915
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Please replace the paragraphs on page 32, lines 3-6, with the following paragraphs
(noting that a portion of the sequence in each of lines 3 and 5 was *underlined in the original*):

GT-Up: 5' TCCGGATCCAATTCAGGAGGTTTTTATGAACAGCAA

TAAAGAGTTAATGCAG (*Bam*HI) (SEQ ID NO:17)

GT-Dn: 5' GATTCTAGATAGGAGCGGCGCTACTGCTTCGCC (*Xba*I) (SEQ ID NO:18).